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Does smoking reduction result in reduction of biomarkers associated with harm? A pilot study using a nicotine inhaler

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The aim of the study was to determine if smoking reduction using a nicotine inhaler in heavy cigarette smokers who wanted to reduce but not stop smoking results in decreased levels of known biomarkers of harm. The study design was a one-sample within-subject comparative open-label study of 23 (10 male and 13 female) subjects using a nicotine inhaler to reduce smoking, with follow-up at 24 weeks. A structured protocol was used with a smoking-reduction schedule from 40 or more cigarettes per day to 10 cigarettes per day by week 9. Behavioral counseling was provided by a research assistant and *ad lib* use of the nicotine inhaler for 12 weeks was permitted. Blood thiocyanate, cotinine, 4-aminobiphenyl hemoglobin adducts; urine NNAL and NNAL-glucuronide; and expired air carbon monoxide were measured. On average, the subjects were able to reduce their smoking by over 50% at week 12, but only two were able to reduce to 10 cigarettes per day. The reported reduction in smoking was not associated with a consistent reduction in the biomarkers. There was no reduction in the NNAL, 4-aminobiphenyl hemoglobin adducts nor carbon monoxide levels of expired air. There was a significant reduction of NNAL-glucuronide and the sum of NNAL and NNAL-glucuronide but only at week 24. Thiocyanate levels increased. Before widely promoting harm reduction as a treatment strategy for heavy smokers, more research needs to be performed to prove conclusively that such smokers who want to reduce but not stop can actually reduce and maintain their smoking rate at a level which is likely to reduce harm. It also needs to be determined whether a reduction in the smoking rate translates into reduction of harm. At the present, for heavy smokers, an abstinence approach seems to be more scientifically sound.

Introduction

Despite the best available treatment, most smokers are either unsuccessful in their attempts to stop smoking or are unwilling even to try. In recent years, the concept of reducing the harm from cigarettes by smoking a reduced number of cigarettes per day has been introduced (Hughes, 1995, 1998). Further, reduced smoking utilizing nicotine replacement therapy has been promoted as a valid method of reducing tobacco-caused harm in those unwilling or unable to stop smoking (Anderson & Hughes, 2000; Jimenez-Ruiz, Kunze, & Fagerström, 1998). Since the development of tobacco-caused diseases is in part a dose-dependent risk, it is theorized that reduced smoking, i.e., smoking fewer cigarettes per day, may lead to reduced harm. However, it may be difficult to motivate smokers to reduce and maintain their smoking at levels that reduce the tobacco-caused harm. Only a small minority of smokers recruited for the large Community Intervention Trial (COMMIT) for smoking

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cessation was able to reduce and maintain significant reduction in smoking (Hughes, Cummings, & Hyland, 1999). Whether significant reduction can be sustained, especially in heavy smokers, has not been demonstrated.

Many carcinogen-derived biomarkers can be quantified in human tissues or body fluids. NNK [4-(methylnitrosamino)–1-(3-pyridyl)–1-butanone] is a tobacco-specific nicotine-derived nitrosamine that is a potent pulmonary carcinogen in rodents (Hecht, 1996). NNK is extensively metabolized and virtually all of the metabolites are excreted in the urine. Its major metabolite is NNAL [4-(methylnitrosamino)–1-(3-pyridyl)–1-butanol], also a potent lung carcinogen in rodents. In humans, both NNAL and its glucuronide diastereomers (NNAL-gluc) are excreted in the urine with the latter generally predominating. NNAL-gluc is thought to be a detoxification product of NNAL, and if carcinogenic, it is less active than either NNK or NNAL (Carmella, Akerkar, Richie, & Hecht, 1995). The sum of the NNAL and NNAL-gluc levels provide an estimate of NNK exposure. The within-smoker differences in the ratio of NNAL-gluc to NNAL over repeated determinations are small, but this ratio has been shown to vary at least 10-fold between smokers. This ratio may provide a measure of detoxification potential upon exposure to NNK. Thus, smokers with a higher NNAL-gluc to NNAL ratio may be partially protected from the carcinogenic effects of NNAL and NNK compared to those with a lower ratio (Hecht, 1996; Carmella *et al.*, 1995).

NNAL and NNAL-gluc are slowly cleared from the body after an individual stops smoking. After a week of abstinence from smoking, the NNAL and NNAL-gluc levels were about a third of pre-cessation levels and after 6 weeks were less than 10% of levels while smoking (Hecht *et al.*, 1999). No effects of nicotine patch therapy on NNAL or NNAL-gluc was observed in these abstinent smokers indicating that NNK is not formed endogenously from nicotine. Since NNAL and NNAL-gluc are based on metabolic transformation of established lung carcinogens, they may provide information about the potential reduction of harm in smokers who reduce their smoking.

Tobacco smoke also contains the aromatic amine 4-ABP (4-aminobiphenyl), a known rodent mammary carcinogen and a human bladder carcinogen (Mooney *et al.*, 1995). The hemoglobin adduct of 4-ABP (4-ABP-Hb adducts) has been shown to correlate with recent smoking and is a measure of passive smoke exposure (Bryant, Skipper, Tannenbaum, & Niure, 1987; Crawford *et al.*, 1994; Maclure, Bryan, Skipper, & Tannenbaum, 1990). The 4-ABP-Hb adducts levels decline over a period of 9–11 weeks after a smoker stops smoking, with a half-life of about 7–9 weeks (Maclure *et al.*, 1990; Mooney *et al.*, 1995).

Other markers of harm from tobacco smoke that are more commonly measured are carbon monoxide (CO) in expired air and thiocyanate levels in body fluids (Vogt, Selvin, & Hulley, 1979). CO levels in expired air are commonly used in clinical trials to biochemically validate self-reported smoking status in a sedentary person. The half-life of CO is about 4–6 h but may be much less with vigorous activity. Hydrogen cyanide is present in high concentrations in tobacco smoke and is metabolized to thiocyanate which can readily be measured in the blood, saliva, or urine of smokers (Velicer, Prochaska, Rossi, & Snow, 1992). The half-life of thiocyanate after a smoker stops smoking is relatively long (10–14 days). Despite its low sensitivity for detecting light smokers, the long half-life of thiocyanate makes it a potentially useful test in determining harmful exposure to tobacco smoke (Jarvis, Tunstall-Pedoe, Feyerabend, Vesey, & Saloojee, 1987). The specificity of thiocyanate is also adversely affected by the large number of food products (broccoli, almonds, beer, cauliflower, etc.) which can cause elevated levels (Velicer *et al.*, 1992).

Thus, for those smokers who are not able to achieve abstinence from smoking, is reduction in their smoking rate a viable alternative? Certainly reduction in tar deliveries (Federal Trade Commission method) by the cigarette manufacturers failed to reduce the all-cause mortality caused by cigarettes (Thun, Day-Lally, Calle, Flanders, & Heath, 1995), and mistaken beliefs about low tar/low nicotine brands reduced the intention of smokers to try to stop smoking (Kozlowski, Goldberg, Yost, White, Sweeney, & Pillitteri, 1998). The primary manufacturing technique used by the cigarette manufacturers for reducing tar and nicotine delivery is using ventilation holes in the filters of cigarettes and not removing harmful chemicals from the tobacco or those formed during combustion of the tobacco. The presence of known potent carcinogens such as benzo(a)pyrene continue to be delivered in cigarette smoke even in the low tar/low nicotine brands (Denissenko, Pao, Tang, & Pfeifer, 1996). Widespread use of FTC measured reduction in tar yield of cigarettes may have a small effect in reducing the risk of cancer (NIH, 1996) but in recent years has perhaps been the etiology of a shift to a higher percentage of adenocarcinoma as a portion of all lung cancers (Levi, Franceschi, La Vecchia, Randimbison, & Te, 1997).

When given the choice of using the various nicotine replacement therapies for smoking reduction, smokers who are motivated to reduce but not stop smoking can reduce their smoking rate over the short term (i.e., 5 weeks). In a study of 143 such smokers, self-reported cigarette consumption was reduced and carbon monoxide levels decreased accordingly, although cotinine levels remained steady suggesting that these smokers were smoking to maintain their nicotine levels while using the nicotine replacement products (Fagerström, Tejding, Westin, & Lunell, 1997). This short-term reduction in the smoking rate using nicotine replacement therapy was also thought by some to increase the smoker's motivation to stop, perhaps by allowing the smokers to gain confidence and increase control over

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their smoking behavior. Whether such reduction using a nicotine replacement product will reduce known biomarkers of harm has not been determined.

This led us to hypothesize that the use of a nicotine inhaler, if successful for smoking reduction, may result in the reduction of known biomarkers of harm such as 4-ABP-Hb adducts, thiocyanate, CO, and urine NNAL and NNAL-glucuronide. Though these markers decline with abstinence from smoking, it is less clear whether they will be significantly reduced with reduced smoking. With reduction, many smokers may compensate just as they do when they switch to low tar/low nicotine delivery cigarettes. The aim of this study was to determine if a protocolized smoking-reduction schedule with ad lib use of a nicotine inhaler in smokers who wanted to reduce but not stop smoking results in decreased levels of these known biomarkers of harm.

Methods

The study design was a one-sample, within-subject comparative, open-label study of 23 heavy (≥40 cigarettes per day) cigarette smokers using a nicotine inhaler for 12 weeks with follow-up out to 24 weeks. The protocol was approved by the Mayo Clinic Institutional Review Board. Subjects who were 18 years of age or older and interested in reducing but not stopping smoking were recruited by press releases and advertisements in the media. Sufficient motivation for smoking reduction was determined by their response to the question, 'Are you motivated to reduce the number of cigarettes you smoke at this time?' Other inclusion criteria included current smoking rate of at least 40 cigarettes per day for the past year and having been a regular smoker for 10 years or more, provision of written informed consent, either postmenopausal status for women or use of an effective form of birth control, and in general good health as determined by medical history and a screening physical examination. Exclusion criteria included pregnancy or lactation; current use (within the past 30 days) of nicotine or tobacco products other than cigarettes; current use of any behavioral or pharmacological smoking cessation program; unstable angina or myocardial infarction within the past 3 months; active (in the previous 12 months) non-nicotine substance dependence; current use of major psychotropic drugs; excessive exposure to fumes, non-tobacco smoke, or environmental tobacco smoke; and the use of antiepileptic medications including phenytoin and phenobarbital (hepatic enzyme induction could result in increased metabolism of some of the biomarkers).

Subjects successfully passing the initial telephone-screening interview made an appointment for an initial clinic visit where informed consent was obtained and demographic, smoking, drug and medical history were obtained. The Fagerström Test for Nicotine Dependence was administered (Heatherton, Kozlowski, Frecker, & Fagerström, 1991), baseline CO levels of expired air were obtained and a brief medical history and physical examination were performed. Subjects who passed all of these screening procedures were enrolled. Blood samples were obtained for thiocyanate, cotinine, 4-ABP-Hb adduct assays and pregnancy tests for women of childbearing potential. Urine samples were collected for NNAL, NNAL-gluc and creatinine assays.

Subjects received a 1-week supply of nicotine inhaler cartridges along with behavioral modification intervention materials that emphasized the importance of behavior change. The intervention and materials were developed specifically for a smoking-reduction strategy and included sessions on motivations for reducing smoking, techniques for reducing smoking, relaxing and managing stress, etc. Each visit included approximately 10–15 min of behavioral counseling by a very experienced research assistant. In order to assure consistency of the behavioral intervention, instructions for and monitoring of inhaler use, and urging to adhere to the cigarette-reduction scheme, the subjects were primarily seen by one research assistant. Subjects were asked to use at least six, but no more than 16, nicotine inhaler cartridges per day. When used properly, each cartridge can deliver approximately 1 mg of nicotine/80 puffs and up to 5 mg/cartridge. Subjects were instructed in the following cigarette-reduction schedule: weeks 1–4, reduce smoking by the end of this period to 30 cigarettes per day; weeks 5–8, to 20 cigarettes per day; and weeks 9–24, to 10 cigarettes per day. Subjects were seen weekly during weeks 1–12, had follow-up telephone calls at weeks 16 and 20, and were seen for a final visit at week 24 during which time all of the blood and urine tests were repeated.

Subjects collected their first morning voided urine sample before breakfast at baseline and at weeks 8 and 24 for analysis of NNAL, NNAL-gluc and creatinine. Plasma samples for thiocyanate and cotinine were collected at baseline and at weeks 4, 8, 12 and 24, while blood samples for 4-ABP-Hb were collected at baseline and at weeks 12 and 24. Analysis of the urine samples (for NNAL, NNAL-gluc and creatinine) and blood samples (for thiocyanate and red cells for 4-ABP-Hb) were performed at the American Health Foundation, Valhalla, New York. NNAL and NNAL-gluc were analyzed in the urine as described by Carmella, Akerkar, and Hecht (1993) and Carmella *et al.* (1995). Plasma thiocyanate was determined by the method previously described by Westley (1987), and 4-ABP-Hb adducts were determined by the method as described by Mooney *et al.* (1995). Plasma cotinine levels were measured at Mayo Medical Laboratories by HPLC (Machacek & Jiang, 1986).

Daily diaries were obtained at baseline and completed through week 12. The daily diaries contained information about amount of daily smoking, withdrawal symptoms and use of the nicotine inhaler. CO levels of expired air were measured at each visit, and a self-reported smoking rate was recorded.

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Statistical methods

Self-reported smoking from the daily diaries and at the weekly visits were used to assess cigarette consumption during the study. Missing smoking rate values were imputed using the carry-forward and carry-backward methods. During weeks 1 through 12, missing diary smoking information for a day in the 7 days prior to a weekly visit was imputed by carrying back the daily smoking rate self-reported at the weekly visit. Smoking data reported at a weekly visit was carried back at most 7 days. Missing smoking data for any extra days between visits (i.e., more than 7 days prior to a visit but after the most recent visit) was imputed by carrying forward the previously reported value. Self-reported smoking rates were summarized weekly as mean cigarettes per day over the past 7 days. The smoking rates were compared to the targeted smoking rates at the end of each reduction phase and the mean change from baseline was compared to zero using the one-sample signed-rank test.

Expired air CO levels were measured at each weekly visit and plasma cotinine was measured at baseline and weeks 4, 8, and 12. The mean changes from baseline in CO and cotinine levels were compared to zero using the one-sample signed-rank test.

Nicotine withdrawal symptoms were assessed daily through the first 12 weeks with a composite withdrawal score computed as the mean of the eight nicotine withdrawal items included on the daily diary (Hughes & Hatsukami, 1986). Symptoms of nicotine withdrawal included desire to smoke; depressed mood; awakening at night; anger, irritability, or frustration; anxiety or nervousness; difficulty concentrating; restlessness or impatience; and hunger. The severity of each symptom over the past 24 h was scored by the subject on a five-point scale as none (0), slight (1), mild (2), moderate (3) or severe (4). For each subject, a baseline withdrawal score was calculated using data from all diaries completed prior to the baseline visit. Withdrawal scores obtained after the baseline visit were analyzed as a mean score and change from baseline and were summarized daily for the first week and weekly thereafter. The mean change in withdrawal from baseline was compared to zero using the one-sample signed-rank test. Also recorded on the daily diaries were the number of nicotine inhaler cartridges used. For each subject, the mean daily number of inhaler cartridges used was calculated on a weekly basis for each of the 12 weeks of the inhaler phase.

The urine (NNAL and NNAL-gluc, both corrected for creatinine) and blood biomarkers (4-ABP-Hb, thiocyanate and cotinine) of interest were summarized as mean levels and changes from baseline. To determine any evidence of a harm reduction, the mean change from baseline for each biomarker level at the scheduled collection weeks was compared to zero using the one-sample signed-rank test. For each subject and biomarker, a separate linear regression model was fit to estimate the relationship between biomarker level and the smoking rate. The biomarker level was the dependent variable and smoking rate was the independent variable. The change in the marker level for a 10 cigarette per day reduction in the smoking rate was estimated for each subject. This rate variable was summarized across all subjects and compared to zero using the one-sample signed-rank test. Only subjects with at least two biomarker level measurements were used. The mean smoking rate was calculated for the past 3 days, 7 days, 4 weeks, and the entire time since baseline to reflect different time intervals prior to the biomarker sample collection. Different time intervals were utilized to investigate whether recent or interval smoking was more predictive of biomarker level. Only the smoking rate over the past 7 days is summarized in the results. We found the results were consistent using the different time intervals.

Additional secondary analyses included assessing the various associations between nicotine withdrawal scores, nicotine inhaler use, CO levels, plasma cotinine levels, and smoking rate. Linear regression models similar to those described above were used to investigate these associations. These statistical methods were also applied to assess associations between the biomarkers, to assess if the association between biomarker levels and smoking rate was dependent on smoking reduction, and to assess the association between biomarker levels and cotinine, CO and thiocyanate levels (other possible measures of smoking-reduction compliance). In all cases, two-sided p-values less than or equal to 0.05 were considered as evidence of significant findings not attributable to chance.

Results

Subject characteristics at baseline are presented in Table 1. The mean±SD age of the 23 subjects was 48.9±11.9 years and 13 (57%) were female. The mean smoking rate was 41.9±3.2 cigarettes per day, with 16 subjects

Table 1. Baseline characteristics (n = 23)

Characteristic	Mean ± SD	Median, range
Age, years	49.1 ± 11.9	51, 29–73
Gender, n (%) female	13 (57)	
Cigarettes per day	41.9 ± 3.2	40, 40–50
Years of regular smoking	32.2 ± 11.0	34, 16–55
Fagerström Test for Nicotine Dependence	8.4 ± 1.1	8, 7–10

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Table 2. Mean smoking rate and carbon monoxide levels

Time	n	Cigarettes per day[a]				Carbon Monoxide, ppm		
		Target[b]	Mean ± SD	Range	Delta[c] ± SD	Mean ± SD	Range	Delta[c] ± SD
Baseline	23	≥40	41.9 ± 3.2	40.0–50.0	–	30.4 ± 9.0	13.5–48.0	–
Week 4	18	30	28.1 ± 4.6	17.9–36.3	−14.0 ± 5.1[e]	27.1 ± 7.7	14.5–42.5	−2.4 ± 8.2
Week 8	16	20	24.3 ± 7.2[d]	17.0–43.0	−17.9 ± 6.8[e]	28.1 ± 8.1	13.5–40.5	−0.6 ± 7.4
Week 12	16	10	18.2 ± 8.2[d]	9.0–40.0	−24.2 ± 7.4[e]	24.1 ± 8.3	4.0–38.5	−4.7 ± 9.5
Week 24	16	10	26.7 ± 10.8[d]	13.0–45.0	−15.7 ± 11.0[e]	26.0 ± 8.0	15.5–50.0	−2.8 ± 9.1

[a] Mean cigarettes per day for the 7 days prior to the weekly visit.
[b] Targeted smoking rate as specified in the protocol.
[c] Change from baseline (time minus baseline).
[d] Two-tailed $p<0.05$ comparing the actual smoking rate to the targeted smoking rate (signed-rank test).
[e] Two-tailed $p<0.001$ comparing change from baseline (delta) to zero (signed-rank test).

smoking 40, one subject smoking 43, four subjects smoking 45 and two subjects smoking 50 cigarettes per day. The mean years of regularly smoking cigarettes was 32.2±11.0. Sixteen of the 23 subjects (70%) completed the 12 weeks of the inhaler treatment phase. Of the seven subjects terminating early, five withdrew consent (two were too busy to attend the visits, two were unable to reduce and one did not like the inhaler), one was removed due to an illness unrelated to the study drug (early stage of coronary artery disease), and one subject was discontinued from the study because of scheduling difficulties.

Table 2 displays the mean smoking rates (cigarettes per day) and carbon monoxide levels at select time points. For 17% of the days, we were missing smoking data, which we imputed using the carry-forward and carry-backward methods as described above. Throughout the study, subjects had significantly reduced their smoking from baseline with mean levels significantly less than baseline during each week ($p<0.001$ for each week). However, only at week 4 was the overall mean smoking rate not significantly greater than the targeted smoking rate of 30 cigarettes per day. Of the 18 subjects remaining in the study at week 4, only 11 successfully reduced their smoking down to the targeted smoking rate of 30 cigarettes per day. By week 8, only 16 subjects remained in the study, of whom three successfully reduced down to 20 cigarettes. Two of the 16 subjects successfully reduced their smoking down to at most 10 cigarettes per day by the end of week 12, but neither subject was able to maintain this reduction through week 24.

Mean expired air carbon monoxide levels were not significantly reduced from baseline at any measured time-point during the study. Although CO levels were not significantly reduced from baseline, the mean levels were significantly associated with smoking rates ($p = 0.003$ from the signed-rank test comparing the median slope of individual subject regressions to zero). Overall, a 10 cigarette per day reduction in smoking was estimated to result in a median reduction of 1 ppm in CO. Figure 1 profiles the mean CO levels and cigarettes smoked per day for the various weeks of the study. Mean smoking levels at each week are based on the 7 days prior to the weekly visit and only include subjects with a visit and a CO measurement at the end of the 7-day period.

The mean plasma cotinine level at baseline was 355.0±170.7 ng/ml. Plasma cotinine levels were significantly lower than baseline for the 17 subjects with cotinine data at week 8 (mean, 297.2±129.5 ng/ml; delta, −82.4±87.8 ng/ml; $p<0.001$) and the 16 subjects with cotinine data at week 12 (mean 289.2±167.1; delta −88.1±75.6 ng/ml; $p<0.001$). This reduction is in spite of having the nicotine inhaler available for use. Cotinine levels were not significantly reduced from baseline at week 4 (367.9±149.7 ng/ml) or at week 24 (322.1±147.6 ng/ml). Cotinine levels were significantly associated with smoking rate ($p<0.001$, signed-rank test comparing the median slope to zero) during the study. A 10 cigarette per day reduction in smoking was estimated to result in a median reduction of 42 ng/ml in plasma cotinine. Cotinine levels were not significantly associated with CO levels or the number of nicotine inhaler cartridges used.

Overall, subjects used an average of 2.5±2.9 (range 0.0–11.9) nicotine inhaler cartridges per day during the first week of use (data available for $n = 22$), and increased its use to 3.2±4.8 (0.2–21.5) and 3.5±7.0 (0.0–30.8) cartridges per day during weeks 2 ($n = 20$) and 3 ($n = 19$), respectively. By week 4 ($n = 18$), average daily inhaler use was 2.4±2.8 (0.0–10.6) cartridges per day, and by week 12 ($n = 16$), mean inhaler use had declined to 1.1±1.8 (0.0–6.1) cartridges per day. During week 12, only eight of the 16 subjects with data reported using the inhaler. The maximum reported number of nicotine inhaler cartridges used on any given day was 39 by one subject who averaged 30.8 cartridges per day during week 3. No plasma cotinine was drawn at week 3, but at week 4 this subject's plasma cotinine level was 222 ng/ml while smoking 18 cigarettes per day and using 10.6 cartridges/day. Daily use of more than 16 cartridges was reported on only 1% of the total days of documented inhaler use of all subjects. Inhaler use was inversely associated with smoking rate,

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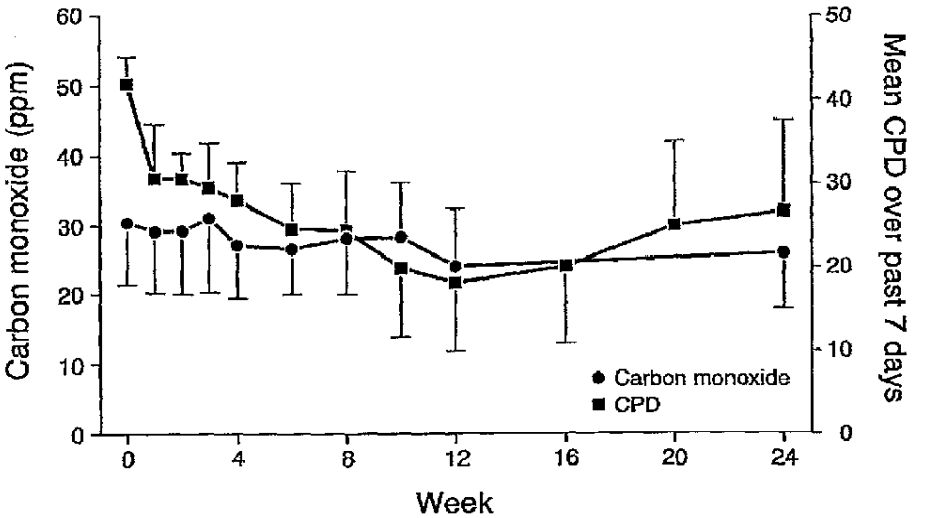

Figure 1. Mean (±SD) carbon monoxide (CO) levels (ppm) and cigarettes per day for various weeks during the study. Mean smoking levels at each week are for the 7 days prior the weekly visit and include only those subjects with CO levels at the following weeks. Mean CO levels were not significantly reduced from baseline but were associated with smoking rate ($p = 0.003$).

with higher inhaler use associated with lower smoking rates. During the 12 weeks of inhaler use, a 10 cigarette per day reduction in smoking was associated with a median increase of 0.4 nicotine inhaler cartridges per day ($p = 0.008$).

Mean nicotine withdrawal scores ranged from 0.9±0.7 at baseline to 1.0±0.6 during week 1, 0.7±0.5 during week 4 and 0.8±0.5 during week 12. Nicotine withdrawal scores were not significantly associated with smoking rate, cotinine levels or the amount of inhaler use. The change in nicotine withdrawal scores from baseline was significant only during week 4 ($p = 0.049$), during which subjects experienced a slight reduction in withdrawal.

Table 3. Mean urine and blood biomarker levels

Biomarker	*n*	Mean±SD	Range	Delta[a]±SD
Thiocyanate, μmol/l				
Baseline	23	189 ± 38	121–282	–
Week 4	17	195 ± 42	139–260	9 ± 30
Week 8	17	211 ± 45	140–284	25 ± 32[b]
Week 12	16	198 ± 37	125–262	15 ± 33
Week 24	16	198 ± 37	160–271	16 ± 19[b]
NNAL, pmol/mg creatinine				
Baseline	18	0.86 ± 0.92	0.31–4.24	–
Week 8	15	0.80 ± 0.67	0.13–2.53	−0.12 ± 0.84
Week 24	15	0.64 ± 0.58	0.30–2.68	−0.28 ± 0.56
NNAL-gluc, pmol/mg creatinine				
Baseline	18	2.83 ± 1.44	0.81–7.56	–
Week 8	15	3.15 ± 1.90	0.99–8.16	0.16 ± 2.31
Week 24	15	2.14 ± 0.99	1.13–5.16	−0.85 ± 0.92[b]
NNAL + NNAL-gluc, pmol/mg creatinine				
Baseline	18	3.69 ± 2.27	1.12–11.80	–
Week 8	15	3.95 ± 2.43	1.12–10.69	0.04 ± 3.08
Week 24	15	2.78 ± 1.54	1.52–7.85	−1.13 ± 1.29[b]
4-ABP-Hb[c], nmol/mol Hb				
Baseline	16	10.4 ± 2.7	7.0–17.2	–
Week 12	15	11.3 ± 4.4	5.6–22.0	0.9 ± 4.6
Week 24	16	9.5 ± 2.5	5.3–14.3	−0.9 ± 2.4

[a] Mean change from baseline (time minus baseline).
[b] Two-tailed $p<0.05$ comparing change from baseline (delta) to zero (signed-rank test).
[c] 4-aminobiphenyl hemoglobin adducts.

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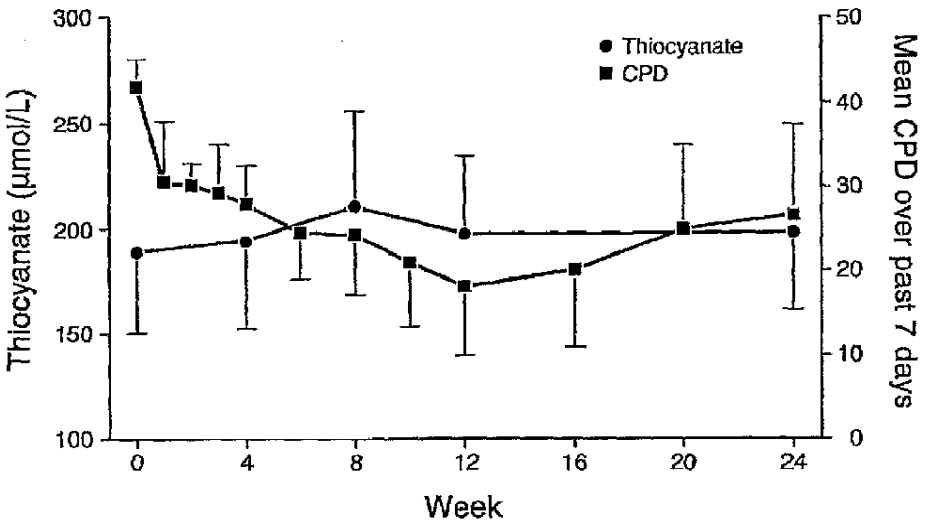

Figure 2. Mean (±SD) thiocyanate levels (µmol/l) and cigarettes per day for various weeks during the study. Mean smoking levels at each week are for the 7 days prior the weekly visit and include only those subjects with thiocyanate levels at the following weeks. Mean thiocyanate levels at weeks 8 and 24 were significantly greater than baseline ($p<0.01$ at both weeks) and the increase in thiocyanate was associated with a decrease in smoking ($p = 0.051$).

Mean levels of the urine and blood biomarkers are presented in Table 3. Mean NNAL indexed to creatinine was not significantly different from baseline at any of the times it was measured. Mean NNAL-gluc and NNAL-gluc levels indexed to creatinine were significantly less than baseline at week 24 ($p = 0.003$). However, the NNAL-gluc levels indexed to creatinine were not significantly associated with smoking rate. Mean NNAL and NNAL-gluc levels indexed to creatinine were significantly associated with each other. Neither of these biomarkers was correlated with CO or thiocyanate. The creatinine indexed NNAL-gluc:NNAL ratio was not significantly associated with smoking rate nor was it significantly different from baseline. However, the sum of the levels of NNAL plus NNAl-gluc corrected for creatinine were significantly reduced from baseline (baseline total, 3.69±2.27 pmol/mg creatinine) at week 24 (mean week 24 totals, 2.78±1.54 pmol/mg creatinine; delta week 24 total, −1.13±1.29 pmol/mg creatinine; $p = 0.004$).

Mean plasma thiocyanate levels were significantly greater than baseline at weeks 8 and 24 ($p = 0.009$ and $p = 0.007$, respectively), with the increase in thiocyanate being marginally associated with the smoking rate ($p = 0.051$; Figure 2). A 10 cigarette per day reduction in smoking was estimated to result in a 7 gmmol/l increase in thiocyanate. Thiocyanate levels were not associated with any of the other biomarkers.

At no time point were the 4-ABP-Hb adducts significantly different from baseline. The 4-ABP-Hb adducts were not significantly associated with any of the other biomarkers. Figure 3 displays the changes (slopes) in thiocyanate, 4-ABP-Hb adducts, NNAL and NNAL-gluc indexed to creatinine, and carbon monoxide per 10 cigarette per day reduction for each individual subject. Each dot represents each individual subject's slope of the regression of the biomarker on mean cigarettes per day for the past 7 days (expressed per 10 cigarette per day reduction).

There was no significant difference in the mean slopes obtained from individually regressing biomarkers against smoking rate when comparing subjects who reduced their smoking to 15 cigarettes per day or less ($n = 8$) by week 12 with subjects not achieving this reduction ($n = 8$). In addition, biomarker levels were not significantly reduced from baseline for either of these groups at any of the time points. Similar results were observed at week 12 when comparing subjects smoking 20 or fewer cigarettes per day ($n = 12$) to subjects smoking more than 20 cigarettes per day ($n = 4$).

Discussion

In this study, we showed that short-term reduction in smoking has mixed results on various biomarkers of harm, and we can draw no definitive conclusions across the biomarkers we tested. Though the reduction in the urine NNAL levels did not achieve statistical significance, there is evidence of reduced exposure to NNK, a nicotine-derived tobacco-specific nitrosamine and a potent pulmonary carcinogen. The conjugated product of NNAL (i.e., NNAL-glucuronide, a presumed detoxification product of NNK) did show a significant decline. Further, there was a significant decline in the sum of NNAL and NNAL-gluc, an important measure of NNK exposure. We observed no reduction in the 4-ABP-Hb adducts which, given a half-life of 7–9 weeks, would

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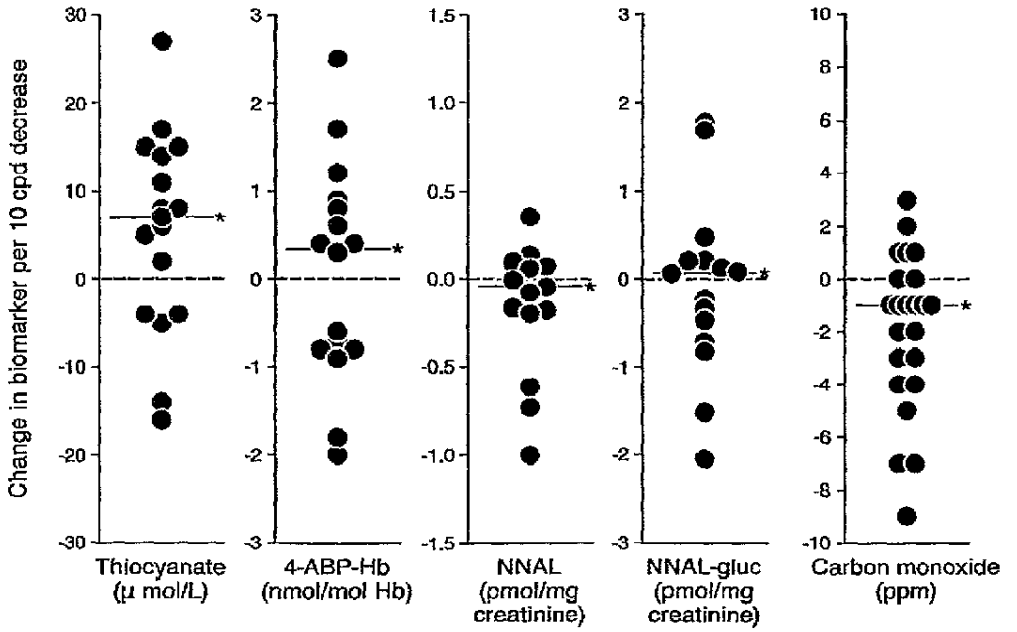

Figure 3. *Changes in biomarker per 10 cigarette per day decrease for each subject. Linear regression models were used to estimate the relationship of the biomarker (y) with smoking rate (x). Each dot represents each individual subject's slope of the regression of the biomarker on mean cigarettes per day over the past 7 days. The rate variable (slope) for each biomarker was summarized across all subjects and compared to zero using the signed-rank test to determine any evidence of harm reduction. A reduction in markers associated with a reduction in smoking was evident in carbon monoxide ($p = 0.003$). * Median change in biomarker level per 10 cigarette per day decrease.*

have been predicted to have declined significantly since there was a reduction in cigarette consumption at week 12 when the hemoglobin adducts were assessed (Mooney *et al.*, 1995). Given the clearance of some biomarkers and the mismatch between the observed reductions in smoking (maximum at 12 weeks) and biomarker measurement (mostly at 24 weeks), it is possible that prolonged reduction in smoking still may have reduced the biomarkers had we followed the subjects for a longer time. Because of the long half-lives of the markers, the measurements taken at 24 weeks may underestimate the true magnitude of decline. In addition, there was no significant reduction in carbon monoxide levels of expired air, which, though not a carcinogen, is a smoking-related toxin. Finally, there was the paradoxical observation that the plasma thiocyanate levels increased with the reduction of smoking in our subjects, and we have no ready explanation for this. As pointed out earlier, several commonly ingested foods can elevate plasma thiocyanate levels, and we did not control for dietary factors in this study. Thus, we have been able to demonstrate that the detoxification product of one biomarker (NNAL) seemed to decrease with an overall reduction in smoking among heavy smokers, but the four other biomarkers did not decline. At this state of our knowledge, we do not know how much reduction in smoking is required to significantly decrease the exposure to toxins nor do we know the duration of reduction required to produce these changes.

The rationale for studying heavy smokers was to maximize our chances of seeing a decrease in the biomarkers of harm. Furthermore, we wanted to study a group of smokers who were as homogeneous as possible on their baseline smoking rate. In this manner, we tried to avoid issues such as: does reducing from 40 to 30 cigarettes per day produce the same relative reduction in the biomarkers that might occur with reducing from 20 to 10 cigarettes per day. Our results highlight the concern that for heavy smokers, a reduction strategy as we proposed may not be appropriate. Our subjects received weekly behavioral counseling and support to achieve their reduction goals, but only a small fraction was able to achieve those goals. Even for these involved and counseled subjects, they were unable to maintain their reduced smoking over the ensuing weeks. While able to achieve a 25% reduction at week 4, they were unable to reach a 50% and 75% reduction to 20 and 10 cigarettes per day at weeks 8 and 12, respectively. Nonetheless, on average, the subjects were able to reduce their smoking by over 50% at week 12, but only two were able to reduce down to 10 cigarettes per day, the target for week 12.

The present outcome is different from the results of the COMMIT trial in several ways (Hughes *et al.*, 1999). First, our smokers were recruited to reduce their smoking rate while the COMMIT trial subjects were recruited with the intent of stopping smoking. Also, our subjects were heavier smokers by an average

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of more than 15 cigarettes per day (42 cigarettes per day vs. 25 cigarettes per day). Even given these two major differences, the COMMIT trial did demonstrate that a small group (21%) of smokers trying to stop could reduce their cigarette consumption (average decrease 7.5 cigarettes per day) and maintain this for 2 years (Hughes *et al.*, 1999). However, it may be unrealistic to expect heavy smokers to do this, even with a highly structured reduction protocol with considerable behavioral counseling. Despite the fact that many subjects reduced their smoking for the first few weeks, there were only two who were able to reduce their smoking to 10 cigarettes per day, and neither was able to maintain that reduction to week 24. Overall, we had a high study completion rate with over 70% completing the 12-week part of the protocol. In general, this group was highly motivated, a characteristic that may be difficult to emulate in the general population of heavy smokers.

We were surprised that on average our subjects did not use more of the nicotine inhaler, though some individuals did use it as instructed. If we had used a delivery device that required little effort on the part of the subject, such as the nicotine patch, the reductions in smoking might have been more dramatic. In the Fagerström study, the inhaler was the least preferred of the five nicotine replacement products that were tested (Fagerström *et al.*, 1997). Further, it could be questioned whether the use of the nicotine inhaler might have promoted continued smoking because of the behavioral similarities. To date there is very little information published about the effectiveness of nicotine replacement therapy when used for smoking reduction (Fagerström *et al.*, 1997). When used properly, the nicotine inhaler can deliver approximately 1 mg of nicotine for every 80 puffs and up to 5 mg of nicotine/cartridge. However, to extract this level of nicotine requires considerable work on the part of the subject. Since plasma cotinine levels were not associated with CO levels or number of nicotine inhaler cartridges used, this suggests many subjects were not adequately using the inhalers, thus may account for the low success rate in reducing. Nonetheless, the maximal reduction in smoking was at 12 weeks, at which time the nicotine inhaler was discontinued with a subsequent increase in smoking rates. It could thus be inferred that continuing the inhaler for a longer period of time might have sustained the smoking reduction for some of the subjects.

Our study differs in many respects from the study by Fagerström *et al.* (1997) for smokers trying to reduce their smoking. First, their subjects were able to use a variety of nicotine replacement products including nicotine gum, patch, nasal spray, inhaler and sublingual tablet. In addition, they were lighter smokers with a mean smoking rate of 22.6 cigarettes per day compared to over 40 cigarettes per day for our group. In their study, the subjects were able to achieve a 50% reduction in smoking rate and an observed similar decrease in expired air carbon monoxide levels, an observation that we were unable to document. Further, the cotinine levels during their study were comparable to their baseline levels, indicating that the smokers were either compensating by smoking the cigarettes more aggressively, or they were able to extract more nicotine from the nicotine replacement products compared to our subjects using the nicotine inhaler. Their nicotine inhaler subjects used, on average, 5.8 inhaler cartridges per day when assigned to that condition. By contrast, in our study, there was a significant decline in plasma cotinine levels and a much lower usage of the nicotine inhaler. Thus, in our subjects, the method of nicotine delivery was not effective in delivering substantial doses of nicotine. Finally, our sample size was much smaller, and those with consistent biomarker data throughout the study ($n = 15$) may have limited our ability to detect significant changes in some of the biomarkers. However, the given sample was large enough to detect significant differences in both directions in some biomarkers (reductions in NNAL-gluc and the sum of NNAL and NNAL-gluc and an increase in thiocyanate).

We suggest that before widely promoting harm reduction as a treatment strategy, more research needs to be performed to prove conclusively that smokers who want to reduce, but not stop, can actually reduce and maintain their smoking rate at a lower level. This may be particularly relevant to heavy smokers. Further research needs to be performed to determine how much smokers compensate by modifying their smoking behavior (deeper inhalation, holding the smoke in the lungs, etc.) when they reduce their smoking rate. Whether a reduction in the smoking rate translates into a reduction in biomarkers of harm, which results in actual reduction in harm, remains a question. Though there is evidence that smoking reduction does not dissuade some smokers from trying to stop smoking, this has not been conclusively studied in heavy smokers (Hughes, 2000). Certainly for heavier smokers, an abstinence approach seems to be more scientifically sound as we know heavier smokers can stop smoking completely using pharmacotherapy and receiving intensive behavioral intervention (Dale, Hurt, Offord, Lawson, Croghan, & Schroeder, 1995). Finally, our study points out some important methodological issues and the relative crudeness of existing measures.

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